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Topographical disorientation after ischemic mini infarct in the dorsal hippocampus: whispers in silence

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Silent focal ischemic mini infarcts in the brain are thought to cause no clinically overt symptoms. Some populations of hippocampal cells are particularly sensitive to ischemic events, however, rendering hippocampal functions especially vulnerable to ischemia-induced deficits. The present study investigated whether an otherwise silent ischemic mini infarct in the hippocampus (HPC) can produce impairments in spatial performance in rats. Spatial performance was assessed in the ziggurat task (ZT) using a 10-trial spatial learning protocol for 4 days prior to undergoing hippocampal ischemic lesion or sham surgery. Hippocampal silent ischemia was induced by infusion of endothelin-1 (ET-1), a potent vasoconstrictor, into either the dorsal or the ventral hippocampus (dHPC and vHPC). When tested postoperatively in the ZT using a standard testing protocol for 8 days, rats with hippocampal lesions exhibited no spatial deficit. Although spatial learning and memory in the ZT were not affected by the ET-1-induced silent ischemia, rats with dHPC stroke showed more returns when navigating the ZT as opposed to the vHPC rats. Comparison of region-specific HPC lesions in the present study indicated that dorsal hippocampal function is critically required for topographic orientation in a complex environment. Topographic disorientation as reflected by enhanced return behaviors may represent one of the earliest predictors of cognitive decline after silent ischemic insult that may be potentially traced with sensitive clinical examination in humans.

Keywords: silent stroke, endotelin-1, hippocampus, ziggurat task, return behavior, spatial navigation, topographical disorientation, early cognitive decline

INTRODUCTION

Silent brain infarctions are silent radiologic abnormalities without overt stroke-like functional symptoms (Vermeer et al., 2007; Kim et al., 2011). Although most silent infarcts initially have no lasting impact on daily life activities, they indicate a greater risk of transient ischemic attacks or major stroke and therefore are of particular clinical interest (Herderscheê et al., 1992; Vermeer et al., 2003; Kim et al., 2011). The cumulative damage caused by multiple silent strokes also may play a key role in the pathogenesis of cognitive and neurobehavioral disturbances (Herderscheê et al., 1992; Masuda et al., 2001; Vermeer et al., 2003; Kim et al., 2011). Despite the lack of overt stroke-like symptoms in silent strokes, it is believed that they are associated with subtle deficits in cognitive function that typically remain unnoticed (Vermeer et al., 2007). Furthermore, the first silent stroke is often followed by gradual deterioration of cognitive function and hippocampal shrinkage (Lopez et al., 2003; Vermeer et al., 2003; Blum et al., 2012). Thus, the hippocampus (HPC) represents a particularly relevant target for pre-clinical studies in silent stroke.

It seems reasonable to expect that the HPC is sensitive to silent ischemic events (Driscoll et al., 2008). Their dense innervation patterns, excitotoxicity and increased calcium entry rates renders CA1 pyramidal cells and hilar interneurons at a relatively greater risk to ischemia-induced neuronal damage compared to the CA3, denate gyrus or other structures (Johansen et al., 1987; Hsu and Buzsáki, 1993). Work in animal models, using endothelin-1 (ET-1), an endogenous potent and long-acting vasoconstricting peptide (Yanagisawa et al., 1988), showed that multiple ET-1 injections into the HPC (Spanswick et al., 2009; Faraji et al., 2011a) are associated with significant and permanent loss of hippocampal tissue and a robust spatial impairment. The HPC is centrally involved in goal-directed spatial navigation (Morris et al., 1982; Sutherland et al., 1982; Astur et al., 2002; Ergorul and Eichenbaum, 2004; de Hoz et al., 2005; Faraji et al., 2008). However, a regionalized process or functional specialization in different parts (left vs. right and dorsal vs. ventral) of the HPC indicates that the HPC may not act as a functionally unitary structure (Moser and Moser, 1998). In rodents, hippocampal function in the dorsal (septal pole) region differs relative to

its ventral (temporal pole) area so that the former mediates learning and memory and the latter anxiety-related emotional behaviors (Bast and Feldon, 2003; Bannerman et al., 2004; Wang et al., 2013). It is important to note that most evidence on the functional segregation between the dorsal and ventral hippocampus (dHPC and vHPC) arises from studies using a water maze task (WT), a wet-land task that imposes a stressful situation and relies on aversive motivation (D'Hooge and De Deyn, 2001; Aguilar-Valles et al., 2005).

The differential role of dHPC and vHPC in spatial navigation suggests that the HPC represents an ideal structure to investigate the symptomatic and structural consequences of hippocampal silent ischemic mini infarcts. Here, we induced silent ischemic mini infarcts by injection of a miniscule ET-1 concentration (Driscoll et al., 2008) into the dHPC and vHPC in rats. Animals were tested in spatial navigation using the ziggurat task (ZT), a dry-land maze for spatial cognition which employs appetitive motivation. The ZT has demonstrated particular sensitivity to spatial cognitive deficits after cerebral infarcts (Faraji et al., 2009). We hypothesized that in spite of the silent nature of the ischemic lesion used in the present study, dorsal and ventral lesions to the hippocampus may produce different behavioral profiles in the distinct procedural and route system of the ZT. The findings provide new insights into distinct behavioral profiles of dHPC vs. vHPC. Our new rat paradigm may be the clinically relevant model for the mechanisms of gradual hippocampal cell loss and cognitive impairments in silent stroke.

MATERIALS AND METHOD

ANIMALS

Twenty male Wistar rats (4 months of age) were housed in pairs at 20 ± 1°C and kept on a 12-h light/dark cycle (light on from 07:00 to 19:00). Animals were randomly subdivided into three groups: dorsal hippocampus (dHPC) lesion, $N = 7$; ventral HPC (vHPC) lesion, $N = 7$; Sham, $N = 6$. Prior to the testing rats were handled for approximately 4–5 min daily for four consecutive days. Animals were tested in the ZT for 4 days pre-lesion, and for 8 days post-lesion. Animals received water *ad libitum* and a restricted diet starting 4 days prior to training and testing in the ZT. Animals were weighed daily and body weight was maintained at about 85% of their initial body weight by providing additional amounts of food in their home cage at least 2–3 h after completion of behavioral testing. All procedures were approved by the Avicenna Institute of Neuroscience (AIN) Animal Care Committee and were carried out in accordance with NIH guidelines.

BEHAVIORAL TRAINING AND TESTING

Ziggurat task (ZT)

All procedures for ZT testing were similar to previously reported (Faraji et al., 2008, 2010, 2011a). The ZT environment is illustrated in **Figure 1**. Prior to behavioral testing, rats were habituated to the ZT environment for 10 min each day for 4 days. In ZT environment animals must use spatial cues (distal and/or proximal) to navigate to the goal ziggurat.

Pre-ischemic spatial performance

Immediately following the last session of habituation, the testing sessions were conducted over 10 trials per day for four consecutive days within the standard version of the ZT. The cycle consisted of alternating *different-goal* or learning days (days 1 and 3) and *same-goal* or memory days (days 2 and 4). On the learning days, the goal ziggurats representing stepped pyramids (either peripheral or central) were located in a new location, and rats were required to find and learn the location of the goal ziggurat in the new place. The goal ziggurats remained in the same place on the memory days. Thus, the rats were required to remember the location they had learned previously. Two sets of ziggurats were defined in the arena. First, "start" ziggurats, located in each corner, and second, the rest of ziggurats or "goal" ziggurats. On the testing days, the rats, released from each starting point, were allowed to explore the environment. One peripheral goal ziggurat was baited with spaghetti for each trial on days 1 and 2. On days 3 and 4, however, rats were rewarded on the central goal ziggurat. During each testing day, rats explored the environment in 10 trials each and from four different starting points at a randomized position. Across trials, the starting location varied among the four corners of the apparatus, and on each trial, animals navigated in the environment for 80 s or until they found the goal ziggurat. Since the location of the goal ziggurat remained constant from trial to trial for every 2 days, the animals had to learn and remember the new locations of the goal ziggurat for every 2 days.

Post-ischemic spatial performance

The procedures for spatial performance assessment after the HPC ischemia were identical to those described in pre-ischemic sessions, except that the testing days were increased to 8 days. Rats were tested in the ZT for 4 days (days 1–4) with peripheral goal ziggurats and for four additional days (days 5–8) with central goal ziggurats. Using peripheral-central ziggurats or mixed procedure (Faraji et al., 2008) in the present experiment, it was assumed that rats needed to change the peripheral strategies to central strategies every 4 days.

In addition to latency (time spent to find either of the peripheral or central goals), path speed (calculated by dividing the path length by the latency), path length, and returns were evaluated. Returns (**Figure 2A**) were characterized by the different pathways animals chose to return to the goal ziggurat in order to accomplish the task. Returns typically refer to the act of localizing and going back to the goal ziggurat during the goal-directed navigation (Faraji et al., 2008; **Figure 2B**). The movements of the animals were recorded and analyzed by HVS Image tracking system. Furthermore, returns were analyzed and tracked by a motiongraph software (SINA motiongraph, V.II, 2011, Tabriz, Iran; **Figure 2C**). A return was characterized by one stop (i.e., speed of 0.0 m/s lasting at least 1 s) followed by creation of a 180° angle towards left or right on the current route.

ISCHEMIC LESION PROCEDURE

Fourteen rats in two groups (dHPC, $N = 7$; vHPC, $N = 7$) received one unilateral infusion of a low concentration of ET-1 (7.5 pmol; Sigma-Aldrich, USA; 0.5 μL in saline solution;